MAJOR ARTICLE

Mother-to-Child Transmission of Human Herpesvirus–8 in South Africa

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To investigate transmission of human herpesvirus (HHV)–8, 2546 mother-child pairs were recruited from rural clinics in South Africa and were tested for antibodies against lytic and latent HHV-8 antigens. The prevalence of antibodies in children increased with increasing maternal antibody titer (lytic, $\chi_1^2 = 26$, and $P < .001$; latent, $\chi_1^2 = 55$, and $P < .001$). HHV-8 DNA was detectable in 145 of 978 maternal saliva samples (mean virus load, 488,450 copies/mL; range, 1550–660,000 copies/mL) and in 12 of 43 breast-milk samples (mean virus load, 5800 copies/mL; range, 1550–12,540 copies/mL). The prevalence of HHV-8 DNA in maternal saliva was unrelated to latent anti–HHV-8 antibody status but was higher in mothers with the highest titers of lytic antibodies than in other mothers (34% vs. 8%; $P < .001$). The prevalence of lytic anti–HHV-8 antibodies in children was 13% (70/528) if the mother did not have HHV-8 in saliva and was 29% (8/28) if the mother had a high HHV-8 load (>50,000 copies/mL) in saliva (odds ratio, 2.6; 95% confidence interval, 1.1–6.2). The presence of HHV-8 DNA in maternal saliva was unrelated to latent antibodies in children. Saliva could be a route of transmission of HHV-8 from person to person, although other routes cannot be ruled out.

Human herpesvirus (HHV)–8, also known as Kaposi sarcoma–associated herpesvirus, is considered to be a necessary cause of Kaposi sarcoma [1, 2]. However, the modes of transmission of HHV-8 are not fully understood. In Europe and the United States, sex between men is the main behavioral risk factor for developing HIV-1–associated Kaposi sarcoma and also appears to be a route of transmission of HHV-8 infection [3–7]. There is some evidence from South Africa and French Guyana of transmission of HHV-8 heterosexually (i.e., the prevalence of the virus is higher among individuals with more sex partners), horizontally, and from a mother to her child [8–11]. HHV-8 DNA can be detected in the saliva of infected individuals, at varying frequencies and at different levels, and has been proposed as an infectious source of virus [12, 13]. Transmission via breast milk is also a theoretical possibility (another herpesvirus, cytomegalovirus, is transmissible via breast milk). To investigate this possibility further, we conducted a case-control study of mother-child pairs in rural South Africa, focusing on potential routes of mother-to-child transmission of HHV-8. The study was conducted in an area where both HIV and HHV-8 are prevalent [14, 15].

SUBJECTS AND METHODS

Hlabisa district is situated in KwaZulu/Natal, South Africa, ~250 km north of Durban. It covers an area of 3729 km^2 and has a population of ~220,000 people, predominantly Zulu [16]. The district has a single government hospital and 15 rural health clinics (staffed by nurses) that are used by almost the entire population; only a minority can afford private health insurance. Between August 2000 and December 2002, we systematically recruited mothers attending any of 6 state-run vaccination clinics with their children. The clinics

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used were all based in the most rural parts of the district and were at least 15 km from a paved road.

Ethical approval for the present study was granted by the University of Natal and the Liverpool School of Tropical Medicine ethical review boards. Signed, informed consent was obtained from all participants in the study. All participants had the study explained to them in isiZulu, by trained Zulu field workers. The information given and consent form signed were in accordance with the guidelines issued by the University of Natal for research projects that include isiZulu-speaking participants. After informed consent and counseling, mothers were interviewed briefly and asked about social, demographic, and health-related factors. Questionnaires were administered by 1 of 6 trained interviewers in the native language of the mother. Children brought for vaccination by someone other than the biological mother were not included in the study. After the interview, spots of blood were collected from both the mother and child via a finger or heel prick onto filter paper (grade 903; Schleicher and Schuell). Saliva samples were collected from both mothers and children (for infants, saliva was collected by use of a pipette). If possible, breast-milk samples were collected from lactating mothers. All samples were stored at −20°C. On a weekly basis, samples were taken to Durban for storage and for HIV testing on 1 of the blood spots. The mother's HIV status was determined by antibody testing by use of a broad-based HIV-1/HIV-2 ELISA (Vironostika; Organon Teknika) and was confirmed by use of another ELISA (Murex Wellcozyme HIV 1+2 GAC-ELISA). Infants were tested for HIV-1 RNA by use of the NucliSens QT assay (Organon Teknika). This assay has a sensitivity of 1600 HIV-1 RNA copies/50 μL of dried plasma. These techniques have been shown to be accurate and reliable for use with subtype C HIV, which is found in South Africa [17]. If the mother wanted to know her HIV status or that of her child, the results were given at a subsequent clinic visit by trained HIV counselors.

Remaining samples of blood, saliva, and breast milk were shipped on dry ice to the Department of Virology at Hannover Medical School (Hannover, Germany). Assays for HHV-8 were conducted by a single investigator in a blinded fashion. An EIA for K8.1 glycoprotein, a lytic-phase antigen, was used to detect HHV-8 lytic antibodies. In European and African populations, this assay has been shown to be both sensitive and specific [18, 19]. The K8.1 EIA has been described in detail elsewhere [18]. In brief, blood spots were eluted in PBS overnight. Then the samples were diluted 1:3 and added to ELISA plates that had been precoated with K8.1 protein. After incubation, a conjugate antihuman antibody was added, and, after a further period of incubation, substrate solution containing nitrophenyl phosphate (Sigma) was added, and the plates were immediately read spectrophotometrically at 405 nm by use of a standard EIA plate reader (Diagnostics Power CP400). To determine positive samples, first, a mean optical density for the 10 negative controls was determined. Samples were scored as negative if their optical density was <5 SD from the mean of the negative samples. Samples with higher optical density were scored as positive. Those whose optical density was 5–7 SD above the mean were scored as "1+," those whose optical density was 8–10 SD above the mean were scored as "2+," and those whose optical density was >10 SD above the mean were scored as "3+." Each set of plates contained 2 positive controls. If these were not scored in the 3+ category, the plate was repeated. Sensitivity and specificity of both the K8.1 EIA and latency-associated nuclear antigen (LANA) assay performed on blood spots, compared with serum samples, was calculated by analyzing 71 matched blood-spot and serum sample pairs from 44 South African hospital patients and 27 HIV-infected men from the United Kingdom. In the present study, the specificity of K8.1 EIA on blood spots, compared with serum samples, was 70%, and the sensitivity was 81%.

Antibodies against HHV-8 LANA were detected by use of an indirect immunofluorescence assay (IFA), as described elsewhere [21]. The assay has been shown to be reliable in testing samples from Africa [20, 21]. In brief, blood spots were eluted overnight in PBS, and slides were coated with permeabalized BCP-1 lymphoma cells, which are latently infected with HHV-8. Eluted serum samples were tested at a dilution of 1:10. The slides were read immediately, by use of a fluorescent microscope, by 2 independent observers. A sample was scored as positive if it showed clear, punctate nuclear staining. Positive samples were further diluted and retested at concentrations of 1:20 and 1:40. Compared with serum samples, in the present study, blood-spot IFA had a specificity of 90% and a sensitivity of 80%.

HHV-8 DNA was detected in saliva by use of a real-time quantitative polymerase chain reaction (PCR) directed against a 171-bp region of the viral open-reading frame K6. The TaqMan probe with a reporter dye at its 5′ end and a quencher dye at its 3′ end had the sequence 5′-(6-FAM) CACCCACCGCCCGTCCAAATC (TAMRA-FAM)-3′. Amplification primers were K6-10 (forward, 5′-CGCCTAATAGCTGCTGCTACGG-3′) and K6-10 (reverse, 5′-TGCATCAGCTGCCTAACCCAG-3′) and were used at final concentrations of 0.4 μmol/L. The hot-start reaction mix and the LightCycler (Roche) were used to monitor the increase in florescence. DNA was extracted from 200 μL of saliva by use of the Qiagen DNA extraction kit, following the manufacturer's instructions, and eluted in 50 μL of water. The quantitative PCR can reliably detect and quantify 30 copies of DNA/PCR and, with the extraction procedure used, this equates to a quantification limit of 1500 genome copies/mL of saliva. HHV-8 DNA was extracted from breast milk as described for saliva, after first removing the lipid fraction by repeat centrifugation.

Data were computerized by trained clerks using Epi Info software (Centers for Disease Control and Prevention), and statistical analyses were conducted by use of STATA software (version 7;

STATA). Odds ratios (ORs) were estimated by use of unconditional logistic regression modeling, with adjustment for maternal age; tests for association used the χ^2 test on 1 *df*. *P* values are 2 sided. Where indicated in the text and tables, analyses were restricted to mother-child pairs in which the child was aged >1 year, to exclude the possibility of detecting passively acquired maternal antibodies against HHV-8 antigens. Similarly, where indicated, analyses were restricted to HIV-seronegative mothers and their children, because the effect of HIV infection on the ability to detect anti–HHV-8 antibodies is unclear.

RESULTS

In total, 2546 mother-child pairs were recruited. Figure 1 shows the recruitment process and the prevalence of antibodies against HHV-8 lytic and latent antigens and against HIV. The seroprevalence of HIV was 28% (705/2520) among mothers and 22% (155/705) among children of HIV-infected mothers. Among mothers, the prevalence of antibodies was 40% (1009/2512) against HHV-8 lytic antigens and 14% (349/2490) against latent antigens; 8% (205) of mothers were seropositive in both HHV-8 assays, 6% (175) had latent antibodies only, 32% (796) had lytic antibodies only, 46% (1176) had antibodies against at least 1 viral antigen, and 54% (1339) were seronegative in both assays. Among children, the prevalence of antibodies was 12% (288/2501) against lytic antigens and 7% (176/2486) against latent antigens; 1% (29) of children were seropositive in both HHV-8 assays, 6% (146) had latent antibodies only, 11% (259) had lytic antibodies only, 18% (434) had antibodies against at least 1 viral antigen, and 82% (2043) were seronegative in both assays.

Figure 2 shows the prevalence of antibodies against HHV-8 lytic and latent antigens for HIV-infected and -uninfected mothers and children, by age group. For HHV-8, the prevalence of lytic antibodies was higher than that of latent antibodies at all ages, and the prevalence of both increased linearly with age (lytic, $\chi_1^2 = 592$, and $P < .001$; latent, $\chi_1^2 = 77$, and $P < .001$). Among children, the prevalence of antibodies against HHV-8 latent antigens did not differ by sex (7% [88/1255] in boys vs. 7% [88/

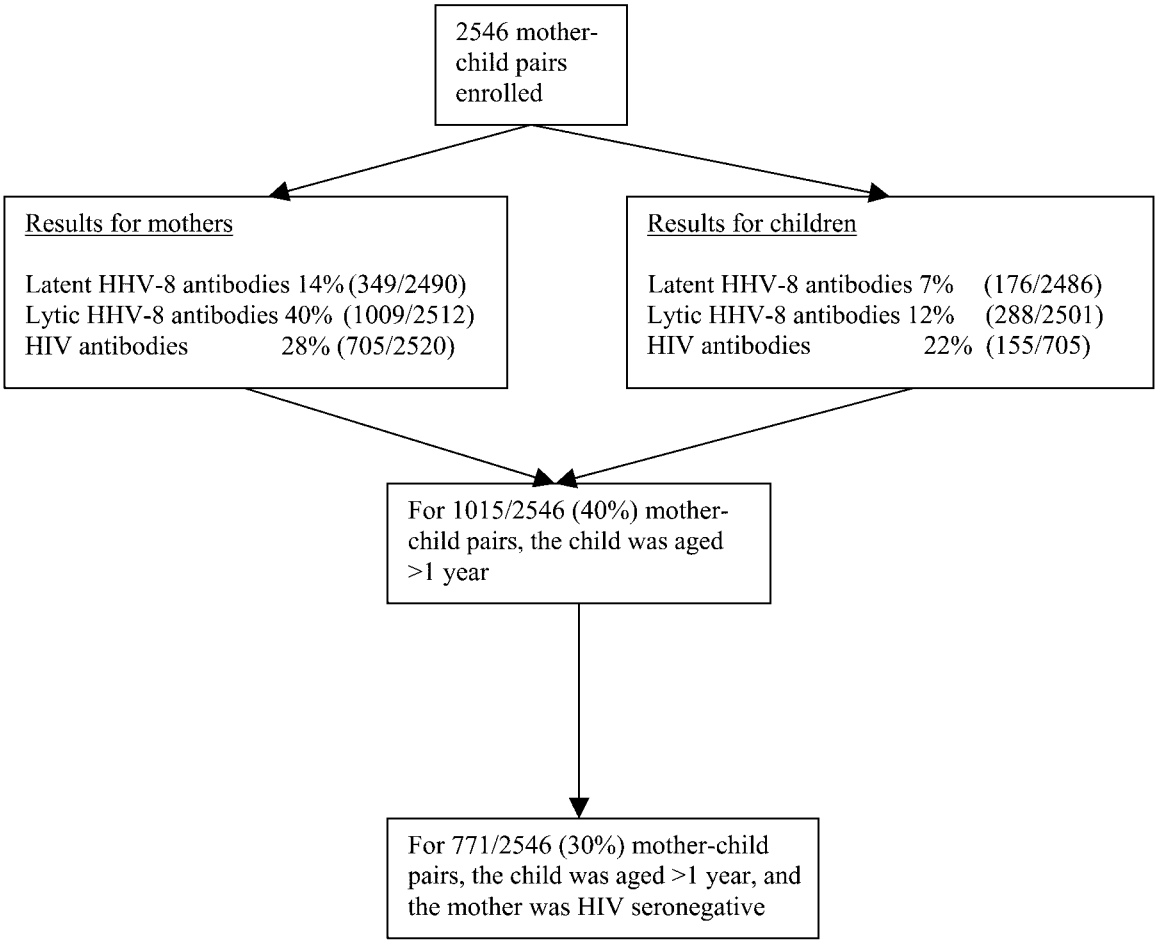

Figure 1. Flow diagram showing recruitment into the study and the prevalence of antibodies against human herpesvirus (HHV)–8 lytic and latent antigens and against HIV in mothers and their children.

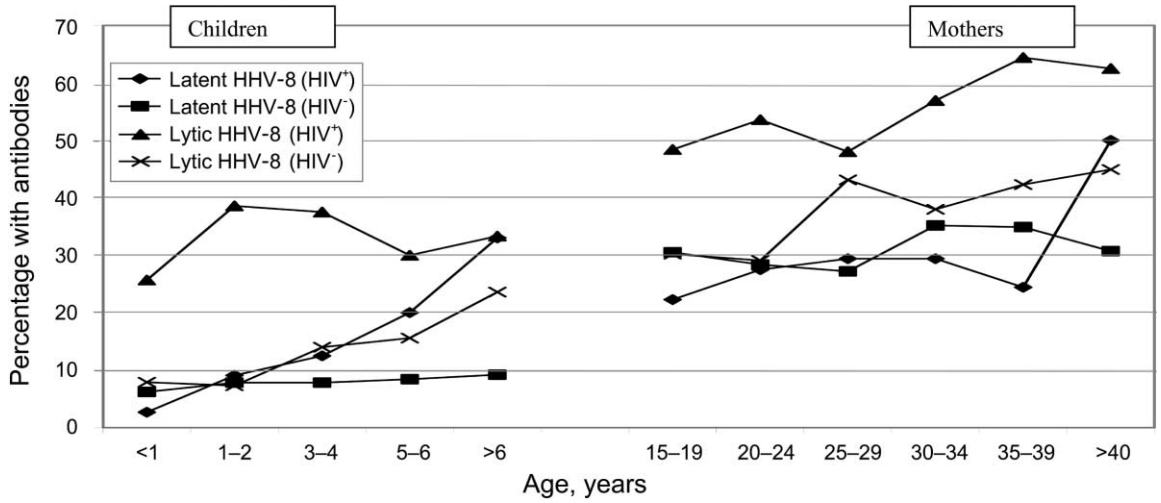

Figure 2. Prevalence of anti–human herpesvirus (HHV)–8 antibodies in mothers and children

1227] in girls; $P = .5$), but lytic antibodies were more prevalent in girls than in boys (13% [160/1234] vs. 10% [127/1263]; $P = .02$). The prevalence of HHV-8 lytic antibodies was 35% (633/1800) in HIV-seronegative mothers and 53% (372/702) in HIV-seropositive mothers (OR, 2.1; 95% confidence interval [CI], 1.7–2.5). In children, the prevalence of lytic antibodies was 10% (237/2297) among HIV-seronegative children and 31% (48/153) among HIV-seropositive children (OR, 4.0; 95% CI, 2.8–5.7). The prevalence of antibodies against HHV-8 latent antigens did not differ significantly between HIV-seronegative and -seropositive mothers (14% vs. 15%; $P = .5$) or children (7% vs. 8%; $P = .5$). The apparent differences seen in figure 2 for specific age groups were not statistically significant.

In children born to mothers with antibodies against HHV-8 lytic antigens, the prevalence of lytic antibodies decreased with age, from 21% (56/272) in children aged 0–3 months, to 12% (18/147) in those aged 4–6 months, and to 9% (7/79) in those aged 7–9 months ($\chi_1^2 = 8.2$, and $P = .004$), presumably indicating decay of passively acquired maternal antibodies. In children born to mothers with antibodies against HHV-8 latent antigens, the prevalence of latent antibodies decreased with age, from 28% (26/94) in children aged 0–3 months to 17% (4/23) in children aged 6–9 months, but the decrease was not statistically significant ($\chi_1^2 = 1.3$, and $P = .3$).

The relationship between maternal antibodies against HHV-8 lytic and latent antigens and presence of antibodies in children

Table 1. Prevalence of antibodies against human herpesvirus (HHV)–8 in children, in relation to the HHV-8 serostatus of the mother (analysis restricted to HIV-seronegative mothers).

		Lytic HHV-8 antibody		Latent HHV-8 antibody	
Age of child, years	Mother's antibody status	Proportion (%) of children with antibody (n = 2482)	OR[a] (95% CI)	Proportion (%) of children with antibody (n = 2452)	OR[b] (95% CI)
<1	Negative	32/712 (5)	1	31/927 (3)	1
	Positive	51/356 (14)	3.6 (2.2–5.6)	25/125 (20)	7.2 (4.1–13)
1–2	Negative	13/220 (6)	1	10/281 (4)	1
	Positive	14/111 (13)	2.3 (1.0–5.1)	14/49 (29)	10.8 (4.5–26)
2–3	Negative	4/59 (7)	1	6/85 (7)	1
	Positive	10/41 (24)	4.4 (1.3–15)	3/14 (21)	3.6 (0.8–17)
3–4	Negative	8/51 (16)	1	3/63 (5)	1
	Positive	5/25 (20)	1.3 (0.4–4.6)	3/12 (25)	6.7 (1.2–38)
>4	Negative	14/117 (12)	1	7/155 (5)	1
	Positive	34/88 (39)	4.6 (2.3–9.4)	11/45 (24)	6.8 (2.5–19)

NOTE. CI, confidence interval; OR, odds ratio.

[a] Adjusted for maternal age and HIV status.

[b] Adjusted for maternal age.

Table 2. Prevalence of anti–human herpesvirus (HHV)–8 antibodies in children, by maternal HHV-8 antibody titer (analysis restricted to children >1 year of age with an HIV-seronegative mother).

HHV-8 antibody, mother's titer	Proportion (%) of children with antibody	OR (95% CI)
Lytic[a]		
0	39/447 (9)	1
1+	18/75 (24)	3.3 (1.8–6.2)
2+	12/55 (22)	2.9 (1.4–6.0)
3+	33/134 (25)	3.4 (2.1–5.7)
Latent[b]		
0	26/584 (5)	1
1:10	10/41 (24)	6.9 (3.1–16)
1:20	9/36 (25)	7.2 (3.1–17)
1:40	12/42 (29)	8.6 (4.0–19)

NOTE. Seven hundred seventy-one children were analyzed for lytic antibody; 703 children were analyzed for latent antibody. Odds ratios (ORs) were adjusted for maternal age. CI, confidence interval.

[a] $\chi_1^2 = 26$; $P < .001$.

[b] $\chi_1^2 = 55$; $P < .001$.

is shown in table 1, restricted to HIV-seronegative mothers and their children. For children of all ages, the risk of having anti–HHV-8 antibodies (both lytic and latent) was higher if the mother was HHV-8 seropositive, rather than HHV-8 seronegative. Furthermore, the proportion of children with detectable antibodies against both HHV-8 lytic and latent antigens increased with increasing maternal antibody titers (table 2: lytic assay, $\chi_1^2 = 26$, and $P < .001$; latent assay, $\chi_1^2 = 55$, and $P < .001$).

The prevalence of antibodies against lytic and latent HHV-8 antigens in children of HHV-8–infected mothers is shown in table 3, according to the HIV serostatus of mother and child. Compared with the situation in which both mother and child were HIV seronegative, maternal HIV infection alone was not associated with an increased risk of either lytic or latent HHV-8 antibodies being detected in the child. However, if the child was also HIV seropositive, the risk of the child having lytic (but not latent) HHV-8 antibodies was significantly increased (OR, 3.2; 95% CI, 1.7–6.1).

In total, 978 saliva samples were selected at random from mothers with children aged >1 year and were tested for evidence of HHV-8 DNA. Fifteen percent (145/978) of mothers had evidence of virus in saliva (mean virus load, 488,450 copies/mL; range, 1500–660,000 copies/mL). The proportion of mothers with detectable HHV-8 in saliva was not significantly related to their HIV serostatus (14% [95/674] in HIV-seronegative mothers and 17% [48/289] in HIV-seropositive mothers; $\chi_1^2 = 1.0$, and $P = .3$). The prevalence of detectable HHV-8 DNA in saliva from HIV-seronegative mothers was unrelated to the titer of antibodies against HHV-8 latent antigens but was related to those against lytic antigens (table 4). The prevalence of HHV-8 in saliva was 9% (34/375) in mothers who were seronegative for HHV-8 lytic antibodies, compared with 34% (42/122) in mothers with the highest titers of lytic antibodies (OR, 5.3; 95% CI, 3.2–8.8). Furthermore, the proportion of children with antibodies against HHV-8 lytic antigens was higher among HIV-seronegative mothers with a high saliva HHV-8 load. Among children of mothers with no detectable HHV-8 in their saliva, 13% (70/528) had lytic HHV-8 antibodies, compared with 29% (8/28) of the children whose mothers had a saliva HHV-8 load of >50,000 copies/mL (OR, 2.6; 95% CI, 1.1–6.2; table 5).

A proportion of breast-milk samples were tested for evidence of HHV-8 DNA. The samples of breast milk were selected from among mothers who were found to have high titers of antibodies against lytic HHV-8 antigens. In total, 12 of 43 mothers had detectable levels of HHV-8 DNA in breast milk (mean virus load, 5800 copies/mL of breast milk; range, 1550–12,540 copies/mL).

DISCUSSION

The prevalence of antibodies against both lytic and latent HHV-8 antigens found in the present study and the linear increase in prevalence with increasing age are broadly comparable to the

Table 3. Prevalence of antibodies against human herpesvirus (HHV)–8 antigens in children of HHV-8–seropositive mothers, according to the HIV serostatus of mother and child (analysis restricted to children aged >1 year).

		Lytic HHV-8 antibody		Latent HHV-8 antibody	
Mother's HIV serostatus	Child's HIV serostatus	Proportion (%) of children with antibody (n = 410)	OR (95% CI)	Proportion (%) of children with antibody (n = 156)	OR (95% CI)
Negative	Negative	63/264 (24)	1	31/119 (26)	1
Positive	Negative	18/100 (18)	0.7 (0.4–1.3)	5/25 (20)	0.7 (0.3–2.2)
Positive	Positive	23/46 (50)	3.2 (1.7–6.1)	4/12 (33)	1.6 (0.4–5.8)

NOTE. Odds ratios (ORs) were adjusted for maternal age. CI, confidence interval.

Table 4. Proportion of mothers with detectable human herpesvirus (HHV)–8 DNA in their saliva, in relation to maternal anti–HHV-8 antibody titers (analysis restricted to HIV-seronegative women with a child aged >1 year).

HHV-8 antibody, mother's titer	Proportion (%) of mothers with HHV-8 DNA in their saliva	OR (95% CI)
Lytic		
0	34/375 (9)	1
1+	6/68 (9)	1.0 (0.4–2.4)
2+	1/46 (2)	0.2 (0.1–1.7)
3+	42/122 (34)	5.3 (3.2–8.8)
Latent		
0	66/506 (13)	1
1:10	4/36 (11)	0.8 (0.3–2.4)
1:20	4/30 (13)	1.0 (0.4–3.0)
1:40	7/38 (18)	1.5 (0.6–3.6)

NOTE. Six hundred eleven mothers were analyzed for lytic antibody; 610 mothers were analyzed for latent antibody. Odds ratios (ORs) were adjusted for maternal age. CI, confidence interval.

findings of other studies from Africa [8, 16, 22–25]. We have confirmed, with considerably larger numbers of subjects than have been studied previously, the increased risk of a child being HHV-8 seropositive if the mother is, together with the increasing prevalence of anti–HHV-8 antibodies in children with increasing maternal antibody titers [10, 11, 26]. This result is suggestive of mother-to-child transmission of the virus. As with other studies from Africa and French Guyana, however, a proportion of children had HHV-8–seronegative mothers and so must have been infected from another source [10, 11, 26]. We did not recruit siblings or fathers of the children and so are unable to comment on these people as possible sources of HHV-8 infection. An increase in the prevalence of HHV-8 seropositivity among children of HHV-8–seropositive mothers was evident in these data only if the child (as well as the mother) was infected with HIV. HIV infection in the mother, but not the child, was not associated with a higher prevalence of antibodies against HHV-8 in the children.

The exact mechanism by which HHV-8 is transmitted is unknown. Epidemiological evidence has pointed to sexual behaviors, such as deep kissing and oro-anal contact, among homosexual men [12, 13]. In Africa, however, where HHV-8 occurs in children, other routes of transmission are likely. HHV-8 DNA has been isolated from saliva samples of HHV-8–seropositive people, but shedding in saliva is intermittent [12, 13]. Here, we found that 15% of mothers had detectable HHV-8 in their saliva. Samples were obtained from the mothers only once, so this is likely to be an underestimate of the number of mothers who might carry HHV-8 virus in their saliva at some point in time. Nevertheless, HHV-8 in saliva was most prevalent in women with high titers of antibodies against HHV-8 lytic antigens (a risk factor for seropositivity in the child) and, at high copy numbers only, was also associated with a higher prevalence of antibodies in the child. These data therefore provide some evidence to support the view that transmission of HHV-8 from person to person might occur through saliva. HHV-8 DNA was also identified in a proportion of breast-milk samples, but at levels that were orders of magnitude below those found in saliva (and well below the levels in saliva that were associated with an increased prevalence of antibodies in the child). However, the volume of breast milk to which a child would be exposed is greater than that of saliva, so it must be considered a potential source of infection. We cannot rule out the possibility that a proportion of children were infected in utero or at birth, although previous research has shown this to be rare [25, 27].

Our finding that a very high virus load in saliva is associated with transmission to the child raises the question of how the child is exposed to maternal saliva. An extensive questionnaire used during this study to collect information on living conditions did not find a clear-cut association with HHV-8 infection in the child (M.D., unpublished data). A recent review describes traditional medicinal practices involving the use of

Table 5. Human herpesvirus (HHV)–8 load in maternal saliva, in relation to the prevalence of anti–HHV-8 antibodies in children (analysis restricted to children aged >1 year with an HIV-seronegative mother).

	Lytic HHV-8 antibody		Latent HHV-8 antibody	
Mother's saliva HHV-8 load, copies/mL	Proportion (%) of children with antibody (n = 610)	OR (95% CI)	Proportion (%) of children with antibody (n = 606)	OR (95% CI)
0	70/528 (13)	1	40/524 (8)	1
1–5000	5/26 (19)	1.6 (0.6–4.3)	0/25 (0)	…
5001–50,000	3/28 (11)	0.8 (0.2–2.7)	2/29 (7)	0.9 (0.2–3.9)
>50,000	8/28 (29)	2.6 (1.1–6.2)	3/28 (11)	1.5 (0.4–5.0)

NOTE. Odds ratios (ORs) were adjusted for maternal age. CI, confidence interval.

saliva that are common in Africa [28]. One example is the use of saliva to soothe itchy bites inflicted by particular insect vectors that could thus promote transmission of HHV-8 ("promoter arthropod" hypothesis) [29].

Our observation that virus shedding in saliva is associated with high lytic, but not latent, antibody titers indicates that levels of antibodies against lytic (structural) antigens may be boosted by active viral replication. High lytic antibody titers may therefore serve as indicators of ongoing viral replication. Conversely, our observations could suggest that HHV-8 antibody levels in individuals who never reactivate and shed HHV-8 could be very low or undetectable. Here, 10% of mothers with undetectable antibodies against the lytic antigen K8.1 and 14% of mothers with undetectable antibodies against LANA had HHV-8 DNA identified in saliva by use of PCR.

The present study has limitations. The case-control design did not allow the incidence of HHV-8 to be determined among the children. In addition, a proportion of the children were <1 year of age and so were excluded from some of the final analyses, because their HHV-8 antibodies may have been maternal. However, our sample was drawn from mothers and children attending state-run vaccination clinics; vaccination coverage in KwaZulu/Natal is >90%, and vaccinations are free, so the study population would have been broadly representative of mothers and children in the area. We used both a lytic and a latent assay to measure HHV-8 infection. The correlation between these assays was poor. Both the lytic and latent assays have been used with African samples before [11, 24, 26, 30, 31]. The use of blood spots on filter paper in the present study may have resulted in a small loss of sensitivity. However, many of our results are in line with those from other studies, suggesting that, at a population level, these assays are performing well enough to examine risk factors for transmission of HHV-8.

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